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SUMMARY

(1) Fifteen earlier introductions of Turkestan alfalfa and 25 lots collected in 1929 by Mr. Westover in six seed-growing districts of Turkestan, together with ten introductions from France and 22 domestic alfalfas were tested for susceptibility to *Aplanobacter insidiosum*.

(2) The 1929 introductions from Turkestan and France and eight domestic alfalfas were also tested for their cold resistance.

(3) Marked differences in wilt and cold resistance of seed lots collected in various seed-growing districts of Turkestan were found.

(4) Provence F. P. I. No. 34886, Hardistan, some of the old and new introductions from Turkestan, and possibly Ladak were found to be resistant to both wilt and cold.

(5) Grimm, together with a number of other varieties and all domestic Common alfalfas tested, was found to be susceptible to wilt.

The Relative Susceptibility of Alfalfas to Wilt and Cold

George L. Peltier, *Plant Pathologist, Nebraska Agricultural Experiment Station,* and H. M. Tysdal, *Associate Agronomist, Office of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [1]

The final solution of the alfalfa-wilt problem in Nebraska probably lies in the development of resistant sorts that are winterhardy as well as productive. The first procedure in the search for such an alfalfa is the testing of existing alfalfas grown in this and other countries. Peltier and Jensen (3) have indicated that three sorts, namely Turkestan, Provence, and Hardistan (2), are quite resistant to wilt, whereas Grimm, Cossack, and other varieties, together with a number of domestic Common alfalfas, are rather susceptible. They had, however, only three introductions of Turkestan and one of Provence under observation, and hence no definite statement of wilt resistance of these sorts in general was made.

During the summer of 1929, Mr. H. L. Westover, Senior Agronomist, Office of Forage Crops and Diseases, U. S. Department of Agriculture, collected a number of different lots of seed in the alfalfa-growing sections of France, eastern Europe, and in various sections of Turkestan. Seed gathered by Mr. Westover, especially from Provence and northwestern France and Turkestan, was supplied for relative-susceptibility tests. A number of domestic alfalfas were also included. The relative susceptibility to wilt of 40 lots from Turkestan, 10 from France, 6 of Grimm from as many states, and 16 domestic alfalfas are reported on at this time, together with hardiness tests of a few well-known varieties and strains and the more recent introductions from Turkestan and France.

RELATIVE SUSCEPTIBILITY TO WILT

METHODS

Seed was planted in January and February, 1930, in the greenhouse, in plant bands (1¾x1¾x2½ inches) placed on top of a 6-inch layer of soil, so that the roots could develop normally and would not be seriously injured when the plants were lifted. The plants were lifted the first week in May, at which time the average root diameter near the crowns was approximately .5 to .75 cm.

One hundred plants of each lot were then prepared for inoculation. The plants, after being lifted, were freed of

[1] This preliminary bulletin is based on co-operative investigations between the Department of Plant Pathology, Nebraska Agricultural Experiment Station, and the Office of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

soil and debris by washing. Care was taken not to injure the roots unnecessarily. A two-weeks-old culture on potato-lactose agar from a recent isolation of *Aplanobacter insidiosum* L. McC. was placed in two liters of water, and the bacterial mass was washed from the agar. The inoculation technique consisted of lightly scraping the roots for a distance of one cm. near the crown with a sharp scalpel, while they were held under the bacterial suspension. They were allowed to remain in this suspension until they were transplanted in the field.

Care was taken to make the wounds on each root approximately the same size and depth so that the portal for the entrance of the bacteria would be as uniform as possible in the different lots under test. Further, the roots were left intact, so that the only visible wounds were those made artificially. Twelve to 24 hours after inoculation the plants were set in the field in rows. A record was made one month after planting, and an occasional plant was found that failed to recover from the effects of transplanting. During the summer the plants were cut twice. The scythe used was disinfected after cutting the plants in each row.

Weather conditions at the time of transplanting were ideal and remained so until July, when extremely hot and dry weather set in and persisted until August. A few plants were beginning to show symptoms of wilt the latter part of June, but the dry weather checked the growth of the plants and no further symptoms were observed until late in September. When the plants were dug in October, top symptoms were evident on many of the plants of the susceptible sorts.

In recording the severity of infection, both top symptoms and the extent of the vascular discoloration in the roots were noted, and the following classification was adopted.

Trace—Discoloration of a few vessels in the inner stele. No top symptoms.

Slight—Discoloration extending in an arc in the inner stele. No top symptoms.

Moderate—Discoloration pronounced and extensive in the inner stele. Slight top symptoms.

Severe—Discoloration extending to the cambium, involving more or less the entire root. Distinct top symptoms.

It was noted that most of the plants had a pronounced growth ring presumably laid down by the plants with the advent of the rains in August, when new growth was resumed.

EXPERIMENTAL RESULTS

In Table 1 are listed the various sorts of alfalfa tested, together with the percentage of healthy and infected individ-

uals. The susceptibility of Grimm to alfalfa wilt has been pointed out by Peltier and Jensen (3), Jones (1), and Salmon (4). However, to determine whether Grimm grown in various localities was equally susceptible, seed lots from five states, Idaho, North Dakota, South Dakota, Nebraska, and Utah, and one from an unknown source were tested. As will be noted from the table, all lots showed a very high percentage of infected plants. The averaged results of the six lots show that only 5 per cent of the plants remained healthy, 20 per cent showed a trace to a slight infection, and 75 per cent were moderately or severely infected. With normal precipitation during the growing season, no doubt many of the plants would have succumbed from wilt in the field.

From the data it appears that the Grimm lots were quite homogenous so far as their susceptibility to wilt is concerned and that irrespective of its origin, Grimm is highly susceptible to wilt under conditions favorable for its spread and development. Cossack, LeBeau, and Hardigan, hardy alfalfas, are equally as susceptible to wilt as Grimm. Likewise, Peruvian, an unhardy vareity, is susceptible.

Altho Peltier and Jensen (3) reported that a number of domestic Common alfalfas were susceptible to wilt, five alfalfas of southern and five of northern origin were included in these tests, to determine whether there were any differences in susceptibility to wilt between strains of varied origin. Under field conditions in Nebraska it has been noted that the stands of southern have deteriorated more rapidly than those of northern alfalfas, and the differentiation between the effects of wilt and winter injury under these conditions has not been definitely determined. The results presented in Table 1 show that all Common alfalfas tested, irrespective of their origin, are susceptible to wilt, altho a few sorts do not show as high a susceptibility as others. This fact may be accounted for in part as follows. The seed listed as "Nebraska" is grown primarily in Sioux, Dawes, and Sheridan counties in the northwestern part of the state. Some of the fields are old and the original source and history of the seed are unknown. Without question in a number of these old fields the plants are more or less resistant to wilt and when the seed is assembled there is included some seed from these old wilt-resistant plants. In other words, Common alfalfa may consist of a mixture rather than one sort. That such incidents occur is known, in that resistant lines have been selected from a number of old fields in Nebraska, the seed of which was mixed with seed from nearby fields and sold as Common alfalfa.

TABLE 1.—*Relative susceptibility of alfalfas to Aplanobacter insidiosum*

Description	F. P. I. or F. C. I. accession Number	Total plants inoculated	Healthy plants	Infected plants			
				Trace	Slight	Moderate	Severe
		Number	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Turkestan	25684	100	76	7	5	11	1
Turkestan	25878	100	72	6	7	15	0
Turkestan	25891	100	70	6	8	12	4
Turkestan	26060	100	69	10	8	12	1
Turkestan	15942	99	64	4	9	19	4
Turkestan	15754	97	64	12	8	15	1
Turkestan	2230	68[1]	63	9	9	19	0
Turkestan	25765	99	63	5	13	17	2
Turkestan	15941	100	60	7	14	13	6
Turkestan	72719	100	59	19	7	12	3
Turkestan	2674	98	58	7	12	17	6
Turkestan (Amtorg)	.....	100	57	8	16	17	2
Turkestan	15809	99	56	3	19	18	4
Turkestan	25392	99	53	11	8	23	5
Turkestan	26263	100	52	11	16	20	1
Turkestan (Tashkent)	84354	97	81	2	7	10	0
Turkestan (Tashkent)	84355	96	80	5	7	5	3
Turkestan (Tashkent)	84383	98	76	9	8	7	0
Turkestan (Tashkent)	84380	99	70	5	7	11	7
Turkestan (Tashkent)	84353	96	63	1	16	17	3
Turkestan (No. East)	84371	71	87	1	3	9	0
Turkestan (No. East)	84367	96	85	3	4	7	1
Turkestan (No. East)	84374	89	80	3	3	4	10
Turkestan (No. East)	84373	93	79	2	11	7	1
Turkestan (Fergana)	84382	81	56	5	16	16	7
Turkestan (Fergana)	84403	87	55	7	16	10	12
Turkestan (Samarkand)	84399	81	85	7	4	4	0
Turkestan (Samarkand)	84401	88	85	3	10	1	1
Turkestan (Samarkand)	84395	91	84	4	8	3	1
Turkestan (Bokhara)	84407	91	85	3	10	2	0
Turkestan (Khiva)	84412	90	86	6	4	4	0
Turkestan (Khiva)	84443	96	84	1	4	9	2
Turkestan (Khiva)	84420	78	82	1	4	13	0
Turkestan (Khiva)	84422	93	77	0	7	10	6
Turkestan (Khiva)	84435	91	71	3	7	12	7
Turkestan (Khiva)	84428	99	69	3	8	13	7
Turkestan (Khiva)	84424	96	66	7	15	9	3
Turkestan (Khiva)	84423	93	64	5	10	14	7
Turkestan (Khiva)	84444	94	56	3	10	23	8
Turkestan (Khiva)	84445	97	51	2	21	20	6
France (Niort)	82602	100	4	4	9	49	34
France (Turenne)	82606	96	4	3	19	36	38
France (Thouare)	82609	98	6	6	16	50	22
France (Provence)	82601	99	7	4	21	35	33
France (Provence)	82615	98	4	6	18	34	38
France (Provence)	34886	50[1]	76	4	6	14	0
France (Provence)	12694	99	1	10	30	38	21
France (Provence)	15743	99	2	3	10	55	30
France (Provence)	15744	100	3	5	16	50	26
France (Sarthe)	15745	40[1]	2	2	13	35	48
Hardistan	.....	98	65	13	10	9	3
Ladak	14135	100	60	4	13	18	5
Grimm[2]	632	100	5	7	10	53	25
Grimm (So. Dak.)	14196	100	3	2	16	55	24
Grimm (Utah)	14464	100	4	5	11	46	34
Grimm (No. Dak.)	14467	98	9	6	13	44	28
Grimm (Idaho)	15936	99	3	10	16	59	12
Grimm (Nebr.)	.....	95	4	3	22	43	28

TABLE 1.—*Relative susceptibility of alfalfa to Aplanobacter incidiosum*—(Continued)

Description	F. P. I. or F. C. I. accession Number	Total plants inoculated	Healthy plants	Infected plants			
				Trace	Slight	Moderate	Severe
		Number	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Cossack	2204	100	6	2	22	39	31
Hardigan	M2621	98	5	1	15	46	33
LeBeau	M2622	100	5	3	23	56	13
Peruvian	437	100	6	0	22	38	34
Arizona	15837	99	1	4	21	24	50
Texas	.....	97	5	3	17	38	37
Oklahoma	.....	99	10	6	7	37	40
New Mexico	14470	100	11	3	15	41	30
California	15880	98	11	1	11	27	50
North Dakota	379	99	3	3	13	53	28
Kansas	435	100	6	2	12	40	40
Utah	15819	98	9	2	20	39	30
South Dakota	.....	98	10	13	16	34	27
Nebraska	.....	100	15	12	16	27	30

[1]Old seed, poor germination.

[2]Obtained from A. Dickinson Co., Chicago, Ill.

Salmon (4) also reports that one sort of Kansas Common is more resistant to wilt than other Common alfalfas found in Kansas. It is very likely, therefore, that wherever old fields have maintained productive yields over a long period of years in wilt-infested regions, resistant individuals can be selected from these fields and seed from these may yield resistant types.

Ladak, a variety of alfalfa recently described by Westover (5), apparently is made up of a number of widely diverse individuals; and while it shows less resistance to wilt than Hardistan, it does contain enough resistant plants to make it a desirable variety for further testing. Both Jones (1) and Salmon (4) have reported Ladak as showing some resistance to wilt.

Peltier and Jensen (3) reported that one introduction of Provence (F. P. I. No. 34886) was decidedly resistant to wilt under field conditions. At the present time two plats of this alfalfa planted in 1922 at the Experiment Station are still outstanding from the standpoint of resistance and hardiness. A similar report has recently been made by Salmon (4), who has had this same Provence under observation in Kansas.

Because of the promise of this introduction, five collections made in 1929 in France (listed first in Table 1) and four previous introductions from France were included, together with Provence F. P. I. No. 34886, in the tests. The results

show that all of the nine introductions tested are very susceptible to wilt. The average for the group shows only 4 per cent healthy, 22 per cent with a trace to slight infection, and 74 per cent moderately or severely diseased. Thus the nine collections of seed originating in France are as susceptible to wilt as Grimm.

On the other hand, 76 per cent of the plants remained healthy, 24 per cent were infected with a trace to a moderate infection, and no severely diseased plants were found in Provence F. P. I. No. 34886. This introduction of Provence differs very markedly in susceptibility to wilt from the others tested. Further, its growth habits are unlike those of the susceptible sorts in many respects, and, as will be noted later, it is superior in cold resistance. Therefore Prevence F. P. I. No. 34886 is so unlike the other Provence introductions in wilt and cold resistance and growth habits that there is good reason to doubt that the seed was grown in France.

Plant selections from the field plats of Provence F. P. I. No. 34886 made in 1927 and increased thru cuttings and seed show a much greater degree of resistance than do plants from the original seed. The behavior of these selections, together with that of other selections from wilt-resistant alfalfas, will be reported at another time. Unfortunately, the origin of this particular introduction is not known, other than that it was purchased from a seed firm as Provence seed and distributed by the Office of Forage Crops and Diseases some years ago. It is also unfortunate that this lot of seed should bear the same name as other highly susceptible introductions from Provence.

As stated previously, Peltier and Jensen (3) had only three introductions of Turkestan under observation, namely Commercial No. 2230, seeded at the Experiment Station in 1922, and two lots of commercial seed planted in 1908 and 1911 in the Platte Valley. These three alfalfas appeared to be resistant to wilt under field conditions. Of the 40 Turkestan alfalfas included in these tests, the first 15 listed in Table 1 were collected from remnants of old introductions and commercial lots of seed imported in recent years. Thus, the lot listed as Amtorg reached the United States in the spring of 1929 and two carloads of this seed was distributed in Nebraska. The tags on this seed indicated that it had been assembled at Tashkent, Turkestan. Of the 25 introductions sent to us by Mr. Westover early in 1930, five were collected near Tashkent, four from northeast Turkestan in the vicinity of Alma-Ata, two from Fergana, three from Samarkand, one from Bokhara, and ten from the region of Khiva, which produces from 80 to 90 per cent of all the alfalfa seed grown in

Turkestan. Thus, these tests included alfalfas from all the main seed-growing districts of Turkestan and represent a geographical distribution in some respects similar to most of the seed-growing sections of the United States.

All of the first 15 lots of Turkestan show some resistance to wilt, as can be seen from Table 1, altho they differ in both percentage and severity of infection. In order to compare the susceptibility of these alfalfas, it is desirable to have a standard or measuring stick. Since the history of Hardistan (2) is known, and the original field is still standing and quite productive after 19 years, it has been chosen as a standard for comparison. Therefore the Turkestans can be roughly grouped for the present into three classes, those apparently more, equally, and less resistant to wilt than Hardistan.

Thus, of the first 15 lots of Turkestan which represent remnants of older introductions or commercial shipments of seed, only one, F. C. I. No. 25684, appears to be more resistant to wilt than Hardistan. The following Turkestans have about the same resistance: Commercial No. 2230, F. C. I. Nos. 15754 and 15942, and S. I. A. Nos. 25765, 25878, 25891, and 26060. The following lots of Turkestan seed appear to be less resistant: Commercial No. 2674, F. C. I. Nos. 15809 and 15941, and S. I. A. Nos. 25392, 26263, and 72719, and Amtorg. Salmon (4) has also reported Commercial No. 2230 resistant to wilt at Manhattan, Kansas.

Of the five introductions from Tashkent, Turkestan, F. P. I. Nos. 84354, 84355, and 84383 appear to be more resistant to wilt than Hardistan, while F. P. I. Nos. 84380 and 84353 are apparently slightly less resistant, when the severity of infection is taken into consideration. The four introductions from Northeast Turkestan, namely F. P. I. Nos. 84371, 84367, 84374, and 84373, are apparently more resistant than Hardistan. Fergana represents one of the southern seed-growing districts in Turkestan, and the two lots, F. P. I. Nos. 84382 and 84403, are much more susceptible than Hardistan, both in the number of susceptible individuals and in the severity of infection. The three introductions from the Samarkand district (F. P. I. Nos. 84395, 84399, and 84401) are all more resistant to wilt than Hardistan, as is likewise the one lot from Bokhara, F. P. I. No. 84407.

From the Khiva district ten lots were tested, and a great variation was found in susceptibility to wilt. Three introductions (F. P. I. Nos. 84412, 84443, and 84420) appeared to be more resistant than Hardistan; five (F. P. I. Nos. 84422, 84435, 84428, 84424, and 84423) are as resistant; and two (F. P. I. Nos. 84444 and 84445) much less resistant to wilt than Hardistan.

These results with various lots of seed from six seed-growing districts of Turkestan indicate that, while they are all more or less resistant to wilt, there are certain lots that are too susceptible to warrant further testing. Therefore, only those lots more resistant than Hardistan will be included in further trials.

RELATIVE RESISTANCE TO COLD

METHODS

There were included in the hardiness tests the same 25 new introductions from Turkestan and the five new introductions from France reported in Table 1, together with a number of well-known domestic alfalfas. The complete details of the methods involved, together with a description of the equipment, are being prepared for publication.

Briefly, the method consisted of growing plants in rows in flats (15x18x6 inches) or in four-inch porous clay pots. A variety of known hardiness was included in each flat for a control. Turkestan F. C. I. No. 15754 was used for this purpose since during the past three years it has consistently shown, in control hardiness tests, a slightly higher degree of resistance to cold than Grimm. The plants were grown under optimum conditions for one month in the greenhouse and then hardened off for a period of two weeks in a greenhouse held at a uniform temperature slightly above 0° C. The plants were transferred from the hardening room directly to the freezing chamber where the flats were exposed to an average temperature of –20° C. for approximately eight hours, and the pots to an average temperature of –14° C. for five hours. After freezing, the flats and pots were removed directly to the warm greenhouse and the surviving plants were allowed to recover.

In all instances the relative resistance to cold was based on actual counts of the plants before freezing and on the number of survivals which recovered from exposures to low temperatures. In Table 2 the relative resistance to cold is tabulated as the mean ratio to the control which was considered as 100. The actual percentage of survivals of each lot was calculated in terms of the control alfalfa for each replication and the ratios thus obtained were averaged. Each replication contained 10 to 30 plants, with a like number of the control. In these tests the introductions from each district of Turkestan and France were grouped as units. The probable errors, calculated by the deviation-from-the-mean method,[1] are included in the table. More extensive tests are now under way to

[1] Hayes, H. K. Control of Soil Heterogeneity and Use of the Probable Error Concept in Plant Breeding Studies. Minn. Agri. Exp. Sta. Tech. Bul. 30. 21 pages. 1925.

determine the relative resistance to cold of the various introductions within each of the districts of Turkestan.

EXPERIMENTAL RESULTS

The large variability indicated (Table 2) by the high probable errors for the new introductions from Turkestan and France does not allow any fine distinction to be drawn. However, the results show that differences in resistance to cold occur in the plants from seed grown in the various districts of Turkestan. The introductions from the Khiva district exhibit a marked resistance to cold, while those from Bokhara and Fergana are quite susceptible. The introductions from the Northeast Turkestan, the Samarkand, and the Tashkent districts are intermediate and perhaps do not differ significantly in cold resistance from the control, Turkestan F. C. I. No. 15754.

TABLE 2.—*Relative resistance of alfalfas to cold*

Description	Number of lots	Number of replications of each lot	Survival ratio to Turkestan 15754
Turkestan F. C. I. 15754	1		100
Turkestan (Khiva)	10	12[1]	109 ±1.8[2]
Turkestan (North East)	4	12	89 ±5.2
Turkestan (Tashkent)	5	12	84 ±3.1
Turkestan (Samarkand)	3	12	80 ±6.4
Turkestan (Bokhara)	1	12	68 ±5.1
Turkestan (Fergana)	2	12	57 ±5.9
France (Provence)	2	3	34 ±2.2
France (North West)	3	3	23 ±1.4
Provence F. P. I. 34886	1	7	115 ±2.9
Hardistan	1	12	115 ±2.7
Ladak F. C. I. 14135	1	25	105 ±3.4
Turkestan Com. 2230	1	3	100 ±1.4
Grimm F. C. I. 15713	1	26	87 ±2.8
Nebraska	1	54	72 ±2.7
Arizona F. C. I. 15837	1	78	26 ±1.3

[1] From 10 to 30 plants in each replication together with an equal number of plants of Turkestan F. C. I. 15754.

[2] P. E. of the mean ratio, calculated by deviation from the mean method.

The recent introductions from northwestern France, as well as those from Provence, are very susceptible to cold. There is a decided contrast between these introductions and the old Provence F. P. I. No. 34886 in their reaction to low temperatures. The old Provence alfalfa shows a marked cold resistance, comparable to that of the Khiva group. Salmon (4) has reported that in Kansas this same alfalfa showed a greater resistance to cold, both in the field and in the controlled freezing tests, than Grimm.

As would be expected from field experience, Grimm, Nebraska Common, and Arizona are resistant to cold in the order named. Ladak shows a slightly greater cold resistance than Grimm, a difference which may be significant. Hardistan shows more resistance than either Grimm or the control, Turkestan F. C. I. No. 15754. It is coincidental that Turkestan Commercial No. 2230 has the same survival ratio as the control.

DISCUSSION

The results of the susceptibility tests show that it is possible to find alfalfas which are resistant to both wilt and cold. The promising ones are, however, few in number. The most noteworthy alfalfa so far reported appears to be Provence F. P. I. No. 34886. Selections from this alfalfa have shown a higher degree of resistance to wilt than the original seed. Hardistan (2), recently described as a new variety, is also outstanding in wilt and cold resistance and small amounts of seed should be available in the near future. In the search for resistant alfalfas, all old fields in wilt-infested sections of the country should be tested, for it appears that some of the plants from these fields may yield resistant types. Ladak, while showing some resistance to wilt and cold, contains a larger number of susceptible individuals than either Provence F. P. I. No. 34886 or Hardistan.

While all lots of Turkestan tested have shown some resistance to wilt, those showing less resistance to wilt and cold than Hardistan are not considered promising. It is interesting to note that some of the Turkestan lots, while quite resistant to wilt, are not nearly so cold resistant as Hardistan. There are, however, a few which are better than Hardistan from the standpoint of both wilt and cold resistance. While the results for the various lots of alfalfa seed from Turkestan are presented individually, it is not intended to stress small differences. However, the results with respect to classification into general groups are considered significant. The actual degree of infection may be expected to vary with the environment under which they are tested.

Judging from the wilt-susceptibility tests and field observations over a period of four years, it is not advisable to plant Grimm, even tho it is resistant to cold, in the subirrigated or irrigated sections of Nebraska. This is likewise true of a number of other varieties and strains. All domestic Common alfalfas so far tested, regardless of their origin, appear to be quite susceptible to wilt.

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